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Perfection of organic dye from *Tectona grandis* (Linn) young leaves methanolic extract for mycological staining

S. Abubakar, A. B. Usman, O. Nnadi, C. Alaku and V. A. Etim
Biotechnology and Genetic Engineering Advanced Laboratory, Sheda Science and Technology Complex, P.M.B. 186, Garki, FCT Abuja, Nigeria
salisuabubakar99@yahoo.com

ABSTRACT
Medical laboratory workers and researchers are constantly exposed to danger from chemicals of synthetic dyes during the tissues and microbial staining work. This research work is aimed at methanolic extraction and perfection of organic dyes from *Tectona grandis* (Linn) young leaves for the single step mycological staining, to trim down the use of allergenic, carcinogenic, expensive and non-environmental friendly synthetic dyes. Comparison of physical properties of the dyes such as conductivity, pH, temperature, and spectrophotometric analysis was made with methyl blue (BDH. chemicals, C.I. 4780, England). The statistical positive value of 0.812, determined the great correlation coefficient of the absorbance of the two dyes. Certain species of fungi viz; *Aspergillus* species, *Mucor* specie, *Curvularia* species, *Tinea* species and *Aureobasidium* species to mention but few were stained using the two different dyes. The fungal structures such as mycelia, sporangium, sprongiospore, conidia, conidiophores and phialides were stained reddish brown with *T. grandis* and stood out well against the pale reddish background. The *T. grandis* dye, thus, provides an alternative substitute to synthetic dye, thereby reducing its health implications.

Key words: - Organic dyes, Synthetic dye, *Tectona grandis*, Health hazard, Wet mount.

INTRODUCTION

A dye can be defined as a highly coloured substance used to impart colour to an infinite variety of materials like textiles, paper, wood, varnishes, leather, ink, fur, food-stuff, cosmetics, medicine, toothpaste, etc. As far as the chemistry of dyes is concerned, a dye molecule has two principal chemical groups, viz. chromophores and auxochromes . The chromophore, usually an aromatic ring, is with the associated colouring property. It has unsaturated bonds such as –C=C, =C=O, –C–S, =C–NH, –CH=N–, –N=N–and –N=O, whose number decides the intensity of the colour. The auxochrome helps the dye molecule to combine with the substrate, thus imparting colour to the latter [1]. Natural dyes can be defined as those colorants (dyes and pigments) obtained from animal or vegetables matter without processing [2]. Various parts of plants such as roots, stems, barks, leaves, fruits and seeds may contain colouring matter which can be exploited. Some plants may have more than one pigment located in different parts and organs of the plant. Many natural dyestuff and stains were obtained mainly from plants and dominated as sources of natural dyes, producing different colours like red, blue, yellow, purple black, brown and a combination of these [3]. The interest in the natural dyes for dyeing and printing of textiles has increased because it is considered eco- friendly, the dyes from natural plant sources are interesting for several reasons, one of them is the brilliant colors of dyes and the degree of toxicity of the dyes is also very low [4]. The natural dyes can be divided into two types, the first type known as the intrinsic dyes which produce the colour very fast just by boiling and do not require any stabilization material, and the second type which need additional chemical material on it in order to make the colour permanent and this type of dyes called descriptive dyes [5].

Tectona grandis Linn, is a large deciduous tree which, under favourable conditions, may reach a height of 30-40 m. In dry habitats, growth becomes stunted and branches, widespread and bushy. On good sites, clear boles of 15-20 m or more can usually be obtained, as lower branches are shaded out. Fluting and buttresses are often found at the base of older trees. The bark is thick, grey or light greyish-brown. The leaves are large, 25-50 cm long and 15-35 cm wide, opposite, elliptic or obovate, the underside grey and densely covered with red glandulous hairs. The flowers are small (6-8 mm in diameter), whitish and bisexual. They appear in large panicles containing up to a few thousand flower buds, which open only a few at a time during the flowering period of 2-4 weeks [6]. Chemical constituents reported in leaves includes: Quinones: Tectoquinone, lapachol, deoxylapachol and its isomer, tectoleafoquinone, anthraquinone – napthaquinone pigment. Steroidal compounds: Squalene, poly isoprene-á-tolyl methyl ether, betulinic acid, tecto grandone, monoterpene, Apocarotenoids: Tectoionols-A, Tectoionols-B. Glycosides: Anthraquinone glycosides Phenolic acids: Tannic acid, Gallic acid, Ferulic acid, Caffeic acid and ellagic acid. Flavonoids: Rutin and Quercitin. *Tectona grandis*, leaves have been also reported to contain carbohydrates, alkaloids, tannins, sterols, saponins, proteins, calcium, phosphorus, crude fiber and also contain dye in different colours such as (yellowish-brown or reddish) [7, 8, 9, 10, 11]. This research work focused on perfecting of organic dyes extracted from

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T. grandis young leaves for single step mycological staining, to trim down using expensive, carcinogenic and non environmental friendly dyes.

T. grandis Taxonomy

Kingdom : Plantae – Plants
Subkingdom : Tracheobionta – Vascular plants
Super division: Spermatophyta – Seed plants
Division : Magnoliophyta – Flowering plants
Class : Magnoliopsida – Dicotyledons
Subclass : Asteridae
Order : Lamiales
Family : Verbenaceae – Verbena family
Genus : Tectona
Species : grandis L. f. – teak
Botanical Name: *Tectona grandis* (linn). [12].

MATERIAL AND METHOD

Study area and Sample collection.

The samples were collected from a farmland in Kwali area council of Abuja Federal capital territory of Nigeria which lies between the latitude of $8^0$25` and longitude $6^0$45` and $7^0$45`E. between the month of July and august, the young leaves were handpicked and brought to the industrial laboratory of Sheda science and technology complex (SHESTCO) in a polyethene bag and spread for drying after removing the mid rib.

Dye Extraction

The dried leaves were processed for the dye extraction using procedure adopted from [13, 14]. The dried leaves were grinded into fine powdery form using pestle and mortar, sieved and stored in a dry bottle. Twenty five grams (25g) of the leaves powder was weighted and placed into a conical flask containing 125ml of Methanol (1:5; w/v). While the methyl blue was prepared according to manufacturer's instructions. Both the mixtures were agitated using rotator shaker (Innova 44, Japan) at 140rpm for 72hrs. Two grams of potassium alum were added in *T. grandis* extract to act as a mordant, then the pH was taken and the dye was mixed by shaking vigorously, the *T. grandis* sample was filtered and the concentrated coloured extract was obtained.

Fungal Culture

The fungal cultures were collected from Department of Microbiology, Bayero University Kano, Kano Nigeria, on a Potato Dextrose Agar (PDA sigma Aldrich) slant and resuscitated them on the same fresh PDA (sigma Aldrich) after incubation for 72hrs before microscopic examination.

Slide Preparation and Microscopy

A drop of *T. grandis* extract (dye) was placed on a cleaned grease free slide, using an inoculating needle. A small piece of fungal mycelia from 72hrs old culture, free from media was removed and transferred to a drop of *T. grandis* dyes on slide and by means of the needle the mycelia were teased. The slide was covered with cover slip carefully to avoid air bubbles; the same procedure was applied using methyl blue dye [14]. The slides were examined first under low power X10, X20 and under high power X40 of Carl zeiss microscope objectives.

Spectrophotometric Analysis

Five (5ml) each from prepared dyes were diluted to six falls and scan at 517nm visible using spectrophotometer (Cecil, C-7500, Cambridge, England). The absorbance of each concentration was recorded. The values were used to plots the spectrophotographic comparison between the two dyes and the correlation coefficient of the two dyes were also calculated using the following formula.

$$r = \frac{n\sum XY - (\sum X)(\sum Y)}{\sqrt{(n\sum X^2 - (\sum X)^2)(n\sum Y^2 - (\sum Y)^2)}} \ldots\ldots (1)$$

Where:
r = correlation coefficient.
n = no of samples.
x = independent variable (Absorbance values of *T. grandis* at wavelengths of 517nm).
y = dependent variable (Absorbance values of methyl blue at wavelengths of 517nm). [15].

RESULT AND DISCUSSION

Table one below shows the comparative Physical parameters of *T. grandis* and Methyl blue dyes the parameters include the colour of the two dyes, red brown and blue for *T. grandis* and Methyl blue respectively, the pH, 6.9 of *T. grandis* dyes is less acidic compared with pH, 5.2 of methyl blue, examine pH is the safety determinant factor of a substance that comes in contact with human skin, despite all the dyes performed the same staining functions but *T. grandis* dye is more harmless than methyl blue dye. The values of the temperature and conductivity of the two dyes also varies, conductivity is a physical property of the substance that make it not easily changeable. The conductivity value of 480mg/l for methyl blue is greater than 112.5mg/m of *T grandis*, Hence the *T. grandis* dyes can easily loose power of its affinity than Methyl blue dyes.

From the spectrophotographic comparison between methyl blue and *T. grandis* dyes shown in figure 3, the two curves shows the R^2 values of 0.9105 and 0.9056 for methyl blue and *T. grandis* respectively, the two dyes revealed the highest absorbance at 2.5 for methyl blue and 1.0 for *T. grandis*. The absorbance values were strongly correlated (r=0.812), but the correlation was not significant at ($P \geq 0.05$). This falls within the visible region of the electromagnetic spectrum and

shows the presence of colour imparting chromophores in the dye extract [16].

The five representative fungi stained with both methyl blue and *T. grandis* in figure 4. Are:- (**A**) *A. species* which shows conidiophores, phialides and conidia. (**B**) Mucor species shows, conidiophores, columella, sporangiospores and sprongium. (**C**)Tinea species shows septate branching mycelium with arthroconidia (**D**) *C. species*, shows septate hyphae, simple Conidiophores, and the conidiospores occurred individually on the conidiophores, with single, double and triple transverse septation and (**E**) *A. pullulans*, shows chains of 1- to 5-celled, darkly pigmented arthroconidia representing the *Scytalidium* anamorph of *Aureobasidium* and the presence of numerous hyaline, single-celled, ovoid-shaped conidia (ameroconidia) which are produced on short denticles. The methyl blue dye stained all the fungal structures blue and stood out well against blue background, while the *T. grandis* have the affinity to stain fungal mycelia, as well as any other fungal features such as sporangiapores, philides and sporangia were stained red brown and stood out well against fairly red brown background.

CONCLUSION

There is concerted effort from researchers globally to cares and protect the environment and human in general from resulted problems of synthetic dyes, and attempt to reduce the resulted wastes from synthetic dyes and its mordants to maintain on healthy environment free from pollution, since some synthetic dyes have been known to cause some hazards to the environment and human health. The result of this research shows strong evidence that methanolic extracts from *T. grandis* young leaves contains natural colored (red brown dyes), which by the literature, natural dyes are neither carcinogenic**,** nor hazardous to environment. The dyes are earth friendly, non-allergic, non-toxic to human body and also possessed some therapeutic values for which it has been used in traditional medicines.

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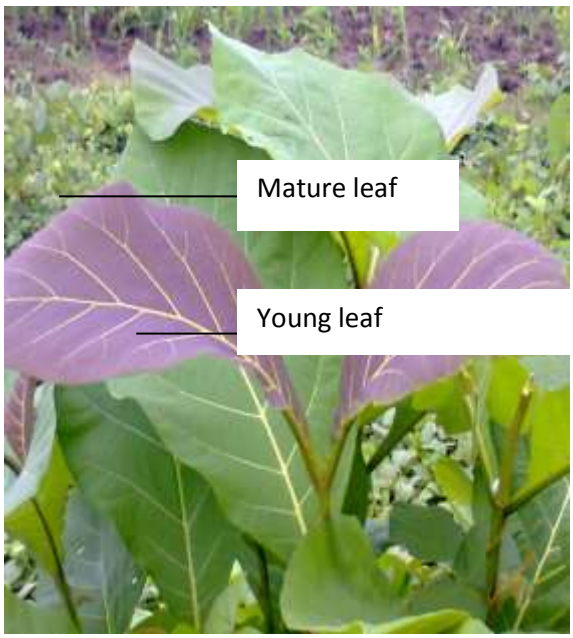

Figure 1: Young *T. grandis* tree (SHESTCO).

Figure 2:- Prepared *T. grandis* and Methyl blue dye.

Table 1:- Physical parameters of *T. grandis* and Methyl blue dyes

Parameters	*T. grandis* Dye	Methyl blue dye
Colour	Red brown	Blue
P.H	6.9	5.2
Temperature	27.8℃	26.4℃
Conductivity	112.5mg/l	480mg/l

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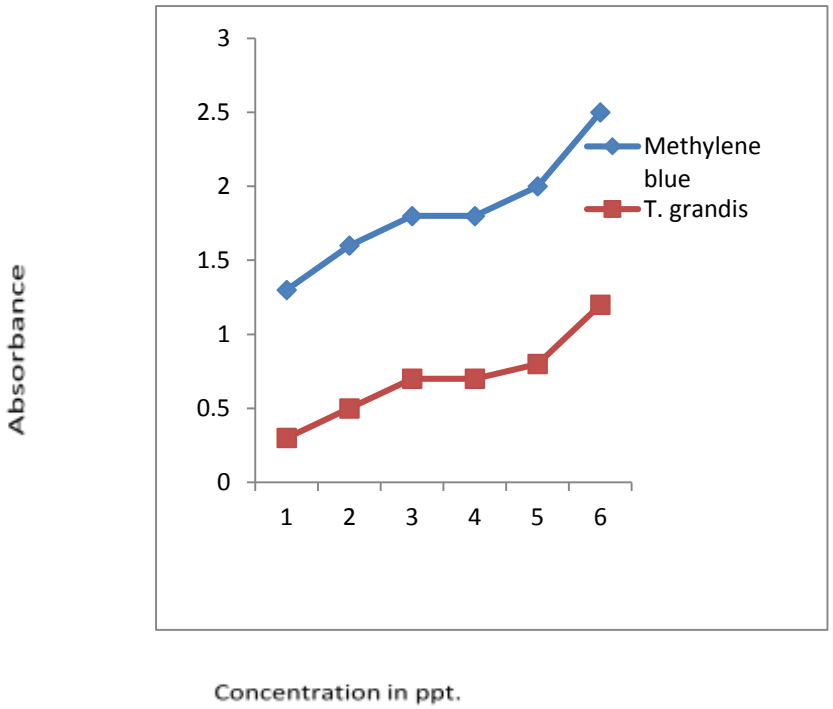
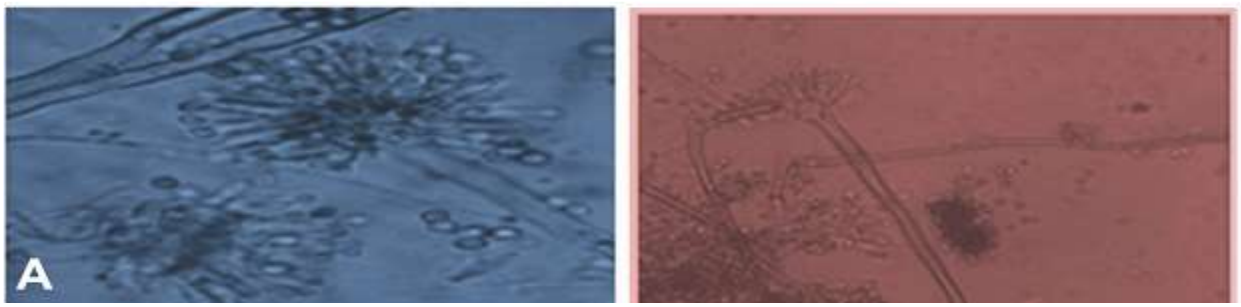


Figure, 3:- Spectrophotographic comparison between methyl blue and T. grandis dyes.



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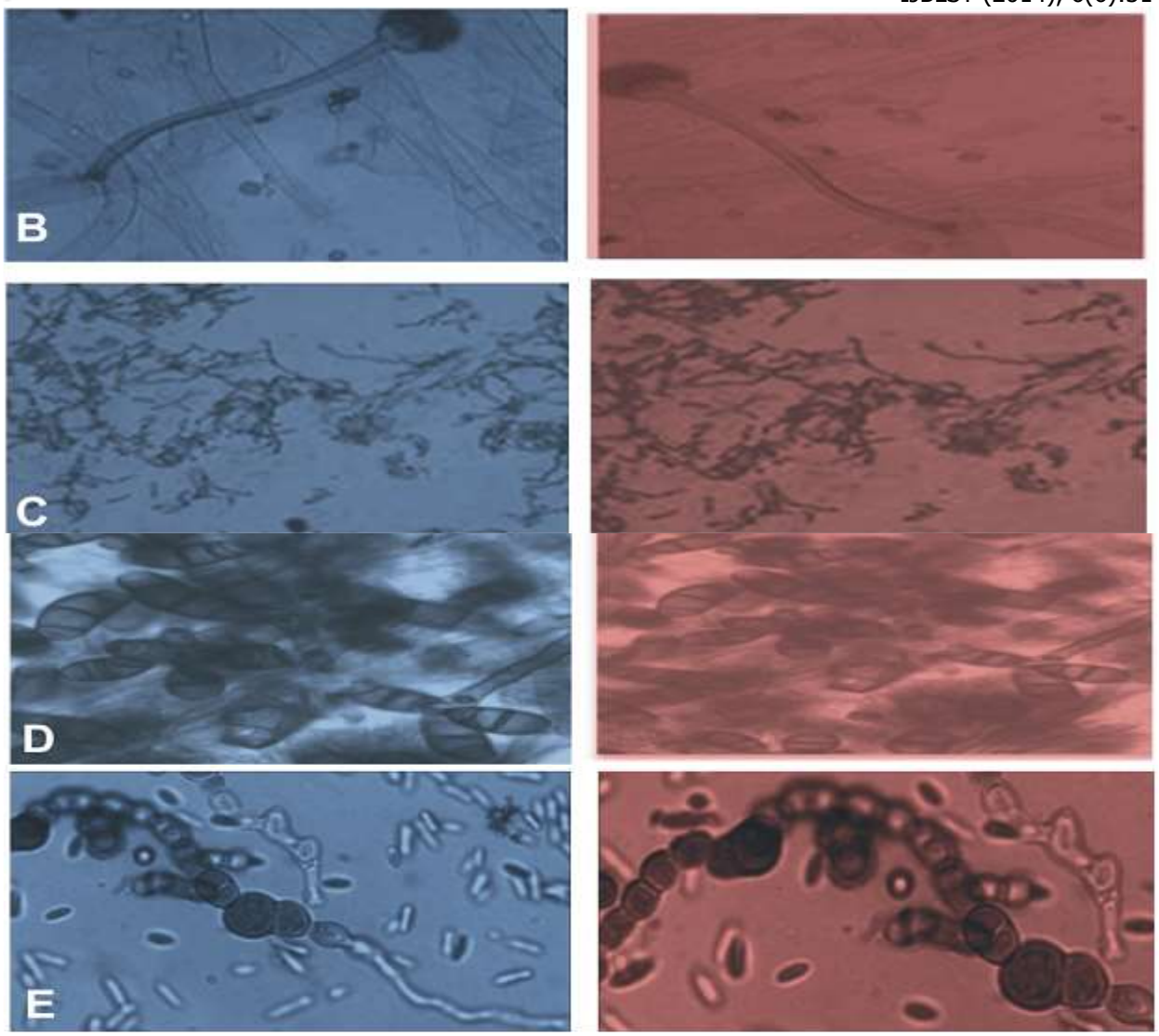

Figure, 4:- {A} *Aspergillus* {B}*Mucor* {C} *Tinea* {D} *Curvularia* and {E} *Aureobasidium sp*.